

EPA Must Revoke the Improperly Granted *Wolbachia Aedes aegypti* Experimental Use Permit

EPA's Office of Pesticide Programs improperly granted an Experimental Use Permit (EUP) to MosquitoMate that permits the release of millions of *Wolbachia*-infected *Aedes aegypti* mosquitoes in California and Florida [1]. Oxitec and other commenters submitted substantive comments on the record identifying significant concerns and critical risks associated with the proposed EUP. Notwithstanding these comments, EPA granted the requested EUP to MosquitoMate. Subsequent to EPA's improvident grant of approval for the release of millions of *Wolbachia*-infected *Aedes aegypti* new data and information has become available that highlights to an even greater extent the potential risks of such releases.

EPA can immediately remedy this improper regulatory approval and all other current *Wolbachia* EUPs. Pursuant to FIFRA Section 5(e) (7 U.S.C. § 136c(e)), the EPA Administrator may revoke an EUP immediately if he finds that its terms or conditions are being violated or that its terms or conditions are inadequate to avoid unreasonable adverse effects on the environment. (See also 40 C.F.R. § 172.10(a)) EPA's regulations provide that a permittee may "contest" the notice of revocation by requesting "an opportunity to confer with the Administrator." The Administrator will issue a final decision within 20 days after such conference. (40 C.F.R. § 172.10(c)) Neither FIFRA nor EPA's regulations provide for a formal hearing to object to revocation of an EUP.

EPA's granting of an experimental use permit to MosquitoMate for an *Aedes aegypti* artificially infected with *Wolbachia pipientis* was premature and did not properly assess the potential adverse impacts to the environment that may result from releases of *Aedes aegypti* artificially infected with a bacterium that is not native to the *Aedes aegypti* microbiome. EPA did not conduct a necessary rigorous assessment of the potential adverse impacts to human health and the environment that could result from uncontained release of *Aedes aegypti* containing this non-native strain of bacteria.

Wolbachia is a complex bacterium that is known to biologically alter its hosts. [5] *Wolbachia* is found in many different insects in nature. Yet that does not make its artificial insertion into *Aedes aegypti* "natural" or "safe". Serious and very basic questions remain as to the potential adverse threats that *Wolbachia* poses to human health and the environment. In cases where *Wolbachia* inhabits insects that bite humans such as mosquitoes and flies, numerous adverse effects have been documented in peer-reviewed publications. Therefore, using *Wolbachia* as a mosquito control solution likely presents very significant risks to public health and the environment.

Some, but by no means all, of the serious health, safety, and environmental concerns regarding *Wolbachia*, include:

- *Wolbachia* holds several key roles in River Blindness which begins with a bite of a blackfly. River Blindness infects up to 25 million people globally and is the second most common infectious cause of blindness; [16]
- *Wolbachia* has been attributed to major lymphatic inflammation associated with Elephantiasis, also known as lymphatic filariasis, which is spread by the bites of infected mosquitoes; [15, 17]

- *Wolbachia* significantly enhances West Nile virus infection in mosquitoes increasing risk of transmission to humans; [8]
- *Wolbachia* enhances malaria parasite infection in mosquitoes increasing risk of transmission to humans; [7, 9, 10, 12] and
- *Wolbachia* has been naturally found in certain mosquitoes (not artificially inserted in a lab), and those infected mosquitoes still are able to transmit dangerous viruses such as dengue and chikungunya. [11]

In addition to these direct and dangerous threats to humans, *Wolbachia* transfers genes to its hosts through a process called Horizontal Gene Transfer (HGT). [2, 3, 4, 5] Thus, artificially infecting *Aedes aegypti* with *Wolbachia* can effectively introduce over 1,000 new genes into its mosquito hosts (as compared to recombinant genetic engineering which typically introduces a few genes or less). This random genetic engineering is not well-defined or understood, yet the result is that every time *Wolbachia* invades a host, or is artificially inserted into a host mosquito, the result is an undefined, randomly genetically modified organism. This stands in stark contrast to precisely genetically engineered organisms, where the genetic transformation is well defined and well understood. Moreover, it appears that *Wolbachia* may be capable of mediating significant lateral gene transfer to higher organisms. [5, 6, 13]

- Peer review papers show that *Wolbachia* genetically modifies its hosts as it transfers its DNA into insect genomes and these genes are expressed;
- *Wolbachia* have high rates of natural DNA genetic change (recombination); [18] and,
- Of significant concern is newly published evidence confirming that *Wolbachia* has a virus that encodes and causes the expression of a toxin from the Black widow spider that is hypothesized to form pores in cell membranes. If mosquitoes that carry *Wolbachia* with this virus are released, the effects of this virus are unknown as are the consequences of these mosquitoes biting people. [14]

Appended is a list of some of the recent publications that provide data and information that raise significant concerns as to the serious adverse public health and environmental effects that may result from release of *Wolbachia*-infected mosquitoes. Some of these effects were well known at the time that EPA granted the *Wolbachia Aedes aegypti* EUP. Others constitute new information regarding potential adverse effects of release of these mosquitoes. EPA should have, but did not, adequately evaluate the known adverse effects of *Wolbachia* at the time the EUP was granted. Moreover, the more recent data and information substantially increase concerns regarding the potential release of millions of *Wolbachia Aedes aegypti*. To date, MosquitoMate has not released large numbers of the *Wolbachia Aedes aegypti* under the approved EUP. However, as is demonstrated in the published literature referenced below, and as Oxitec and other commenters demonstrated on the record of the EUP application, releases of such massive numbers of these artificially infected *Aedes aegypti* could result in serious unreasonable adverse effects to public health and the environment. MosquitoMate must not be allowed to risk these potentially significant adverse effects to public health and the environment.

References

1. USEPA, Amendments, Extensions, and/or Issuances of Experimental Use Permits, 81 Federal Register 69059 October 5, 2016.
2. Woolfit, M., *et al.*, An ancient horizontal gene transfer between mosquito and the endosymbiotic bacterium *Wolbachia pipientis*. *Molecular biology and evolution*, 2009. 26(2): p. 367-74.
3. Horizontal gene transfer between *Wolbachia* and the mosquito *Aedes aegypti*.
[HYPERLINK "<https://www.ncbi.nlm.nih.gov/pubmed/19154594>"]
Excerpt – ***The evolutionary importance of horizontal gene transfer (HGT) from Wolbachia endosymbiotic bacteria to their eukaryotic hosts is a topic of considerable interest and debate...We have discovered a case of HGT, involving two adjacent genes, between the genomes of Wolbachia and the currently Wolbachia-uninfected mosquito Aedes aegypti, an important human disease vector...The data support the argument that HGT between Wolbachia endosymbiotic bacteria and their hosts has produced evolutionary innovation.***
Authors - Klasson L, Kambris Z, Cook PE, Walker T, Sinkins SP
4. A case of horizontal gene transfer from *Wolbachia* to *Aedes albopictus* C6/36 cell line
a. [HYPERLINK "<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4013104/>"]
Excerpt - ***Horizontal gene transfer plays an essential role in evolution and ecological adaptation, yet this phenomenon has remained controversial, particularly where it occurs between prokaryotes and eukaryotes. In this study, we report the discovery of a horizontal gene transfer from the endosymbiont Wolbachia in the C6/36 cell line derived from the mosquito Aedes albopictus. Moreover, we report that this horizontally transferred gene displayed high transcription level. This finding and the results of further experimentation strongly suggest this gene is functional and has been expressed and translated into a protein in the mosquito host cells.***
Authors - Qing Hou , Ji He, Jing Yu, Yuting Ye, Dan Zhou, Yan Sun, Donghui Zhang, Lei Ma, Bo Shen, and Changliang Zhu
5. Widespread lateral gene transfer from intracellular bacteria to multicellular eukaryotes.
a. [HYPERLINK "<https://www.ncbi.nlm.nih.gov/pubmed/17761848>"]
Excerpt – ***Although common among bacteria, lateral gene transfer, i.e., the movement of genes between distantly related organisms, is thought to occur only rarely between bacteria and multicellular eukaryotes. However, the presence of endosymbionts, such as Wolbachia pipientis, within some eukaryotic germlines may facilitate bacterial gene transfers to eukaryotic host genomes. We found and confirmed transfers into the***

genomes of four insect and four nematode species that range from nearly the entire Wolbachia genome (>1 megabase) to short (<500 base pairs) insertions.

Authors - Dunning Hotopp JC, Clark ME, Oliveira DC, Foster JM, Fischer P, Muñoz Torres MC, Giebel JD, Kumar N, Ishmael N, Wang S, Ingram J, Nene RV, Shepard J, Tomkins J, Richards S, Spiro DJ, Ghedin E, Slatko BE, Tettelin H, Werren JH.

6. Genome fragment of Wolbachia endosymbiont transferred to X chromosome of host insect

a. [[HYPERLINK "http://www.pnas.org/content/99/22/14280.full"](http://www.pnas.org/content/99/22/14280.full)]

Excerpt – ***Here we report an unprecedented case of prokaryote–eukaryote horizontal gene transfer: a genome fragment from the Wolbachia endosymbiont has been transferred to the X chromosome of a beetle.....The adzuki bean beetle, Callosobruchus chinensis, is triple-infected with distinct lineages of Wolbachia endosymbiont, wBruCon, wBruOri, and wBruAus, which were identified by their wsp (Wolbachia surface protein) gene sequences. Whereas wBruCon and wBruOri caused cytoplasmic incompatibility of the host insect, wBruAus did not. Although wBruCon and wBruOri were easily eliminated by antibiotic treatments, wBruAus persisted over five treated generations and could not be eliminated...The study's results strongly suggest that wBruAus has no microbial entity but is a genome fragment of Wolbachia endosymbiont transferred to the X chromosome of the host insect.***

Authors - Natsuko Kondo, Naruo Nikoh, Nobuyuki Ijichi, Masakazu Shimada, and Takema Fukatsu

7. Wolbachia Can Enhance Plasmodium Infection in Mosquitoes: Implications for Malaria Control?

[[HYPERLINK "http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1004182"](http://journals.plos.org/plospathogens/article?id=10.1371/journal.ppat.1004182)]

Excerpt - ***Any potential control strategy devised in regions where more than one parasite species occurs needs to thoroughly investigate the effect of Wolbachia on all parasite species transmitted by the vector, as well as other pathogens such as filarial worms or arboviruses to ensure that Wolbachia-infected mosquitoes do not inadvertently enhance transmission of secondary pathogens.***

Authors - Grant L. Hughes, Ana Rivero, Jason L. Rasgon

8. Wolbachia Enhances West Nile Virus (WNV) Infection in the Mosquito Culex tarsalis

a. [[HYPERLINK](http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002965)

"<http://journals.plos.org/plosntds/article?id=10.1371/journal.pntd.0002965>"]

Excerpt – ***This is the first observation of Wolbachia-induced enhancement of a human pathogen in mosquitoes, suggesting that caution should be applied before releasing Wolbachia-infected insects as part of a vector-borne disease control program.***

Authors - Brittany L. Dodson, Grant L. Hughes, Oluwatobi Paul, Amy C. Matacchiero, Laura D. Kramer, Jason L. Rasgon

9. Wolbachia increases susceptibility to Plasmodium infection in a natural system
 - a. [[HYPERLINK "http://rspb.royalsocietypublishing.org/content/281/1779/20132837"](http://rspb.royalsocietypublishing.org/content/281/1779/20132837)]

Excerpt - *These results suggest that naturally Wolbachia-infected mosquitoes may, in fact, be better vectors of malaria than Wolbachia-free ones.*

Authors - F. Zélé, A. Nicot, A. Berthomieu, M. Weill, O. Duron, A. Rivero

10. Wolbachia Strain wAlbB Enhances Infection by the Rodent Malaria Parasite Plasmodium berghei in Anopheles gambiae Mosquitoes
 - a. [[HYPERLINK "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3294472/"](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3294472/)]

Excerpt - *Wolbachia, a common bacterial endosymbiont of insects, has been shown to protect its hosts against a wide range of pathogens. However, not all strains exert a protective effect on their host. We show that the wAlbB strain significantly increases P. berghei oocyst levels in the mosquito midgut while wMelPop modestly suppresses oocyst levels. The wAlbB strain is avirulent to mosquitoes while wMelPop is moderately virulent to mosquitoes pre-blood meal and highly virulent after mosquitoes have fed on mice. These various effects on P. berghei levels suggest that Wolbachia strains differ in their interactions with the host and/or pathogen...*

Authors - Grant L. Hughes, Joel Vega-Rodriguez, Ping Xue, and Jason L. Rasgon

11. Wolbachia in a major African crop pest increases susceptibility to viral disease rather than protects.
 - a. [[HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/22731846"](https://www.ncbi.nlm.nih.gov/pubmed/22731846)]

Excerpt – *Wolbachia have generated considerable recent interest due to the capacity of some strains to protect their insect hosts against viruses and the potential for this to reduce vector competence of a range of human diseases, including dengue. In contrast, here we provide data from field populations of a major crop pest, African armyworm (Spodoptera exempta), which show that the prevalence and intensity of infection with a nucleopolydovirus (SpexNPV) is positively associated with infection with three strains of Wolbachia*

Authors - Graham RI, Grzywacz D, Mushobozi WL, Wilson K.

12. Temperature alters Plasmodium blocking by Wolbachia.
 - a. [[HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/24488176"](https://www.ncbi.nlm.nih.gov/pubmed/24488176)]

Excerpt – *Very recently, the Asian malaria vector (Anopheles stephensi) was stably transinfected with the wAlbB strain of Wolbachia, inducing refractoriness to the human malaria parasite Plasmodium falciparum. However, conditions in the field can differ substantially from those in the laboratory. Our results demonstrate complex effects of temperature on the Wolbachia-malaria interaction, and suggest the impacts of transinfection might vary across diverse environments.*

Authors - Murdock CC, Blanford S, Hughes GL, Rasgon JL, Thomas MB.

13. Phylogenetic relationships of the Wolbachia of nematodes and arthropods.

a. [HYPERLINK "<https://www.ncbi.nlm.nih.gov/pubmed/17040125>"]

Excerpt - *Using the wOvo sequence, we identified a lateral transfer event whereby segments of the Wolbachia genome were inserted into the Onchocerca nuclear genome. This event predated the separation of the human parasite O. volvulus from its cattle-parasitic sister species, O. ochengi. The long association between filarial nematodes and Wolbachia symbionts may permit more frequent genetic exchange between their genomes.*

Authors - Fenn K, Conlon C, Jones M, Quail MA, Holroyd NE, Parkhill J, Blaxter M

14. Eukaryotic association module in phage WO genomes from Wolbachia

a. [HYPERLINK "<http://www.nature.com/articles/ncomms13155>"]

Excerpt - *Here we report a metagenomic analysis of purified bacteriophage WO particles of Wolbachia and uncover a eukaryotic association module in the complete WO genome. It harbours predicted domains, such as the black widow latrotoxin C-terminal domain, that are uninterrupted in bacteriophage genomes, enriched with eukaryotic protease cleavage sites and combined with additional domains to forge one of the largest bacteriophage genes to date (14,256 bp). To the best of our knowledge, these eukaryotic-like domains have never before been reported in packaged bacteriophages and their phylogeny, distribution and sequence diversity imply lateral transfers between bacteriophage/prophage and animal genomes. Finally, the WO genome sequences and identification of attachment sites will potentially advance genetic manipulation of Wolbachia.*

Authors - Sarah R. Bordenstein & Seth R. Bordenstein

15. Onchocerciasis: the Role of Wolbachia Bacterial Endosymbionts in Parasite Biology, Disease Pathogenesis, and Treatment

a. [HYPERLINK "<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3131055/>"]

Excerpt - *Studies with other filarial nematode species have also highlighted a role for Wolbachia in transmission and infection of the mammalian host through a fascinating manipulation of mast cell-mediated vasodilation to enhance infectivity of vector-borne larvae. Wolbachia has also been identified as the principal driver of innate and*

adaptive Th1 inflammatory immunity, which can either contribute to disease pathogenesis or, with the Wolbachia-mediated recruitment of mast cells, enhance infectivity. The Wolbachia activation of innate inflammation also drives inflammatory adverse events in response to chemotherapy with either diethylcarbamazine (DEC) or ivermectin. In this review we summarize the experimental and field trial data which have uncovered the importance of Wolbachia symbiosis in onchocerciasis.

Authors –

16. The Role of Endosymbiotic Wolbachia Bacteria in the Pathogenesis of River Blindness

a. [[HYPERLINK "http://www.nature.com/news/2002/020304/full/news020304-9.html"](http://www.nature.com/news/2002/020304/full/news020304-9.html)]

Excerpt – *Using a murine model for river blindness in which soluble extracts of filarial nematodes were injected into the corneal stroma, we demonstrated that the predominant inflammatory response in the cornea was due to species to endosymbiotic Wolbachia bacteria. In addition, the inflammatory response induced by these bacteria was dependent on expression of functional Toll-like receptor (TLR4) on host cells.*

Authors - Amélie v. Saint André, Nathan M. Blackwell, Laurie R. Hall, Achim Hoerauf, Norbert W. Brattig, Lars Volkmann, Mark J. Taylor, Louise Ford, Amy G. Hise, Jonathan H. Lass, Eugenia Diaconu, Eric Pearlman

17. Wolbachia bacteria in filarial immunity and disease.

a. [[HYPERLINK "https://www.ncbi.nlm.nih.gov/pubmed/11472559"](https://www.ncbi.nlm.nih.gov/pubmed/11472559)]

Excerpt - *Lymphatic filarial nematodes are infected with endosymbiotic Wolbachia bacteria. Lipopolysaccharide from these bacteria is the major activator of innate inflammatory responses induced directly by the parasite. Here, we propose a mechanism by which Wolbachia initiates acute inflammatory responses associated with death of parasites, leading to acute filarial lymphangitis and adverse reactions to antifilarial chemotherapy. We also speculate that repeated exposure to acute inflammatory responses and the chronic release of bacteria, results in damage to infected lymphatics and desensitization of the innate immune system. These events will result in an increased susceptibility to opportunistic infections, which cause acute dermatolymphangitis associated with lymphoedema and elephantiasis*

Authors - Taylor MJ, Cross HF, Ford L, Makunde WH, Prasad GB, Bilo K.

18. Comparative Genomics of *Wolbachia* and the Bacterial Species Concept, [[HYPERLINK](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3616963/)

"<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3616963/>"]

Excerpts - *The importance of host-specialization to speciation processes in obligate host-associated bacteria is well known, as is also the ability of recombination to generate cohesion in bacterial populations. However, whether divergent strains of highly recombining intracellular bacteria, such as Wolbachia, can maintain their genetic distinctness when infecting the same host is not known.*

Finally, we inferred several cases of gene transfers of phages between strains of different supergroups. All genomes contain prophages of similar organization [[HYPERLINK "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3616963/"](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3616963/) \l "pgen.1003381.s005"]] and our single gene phylogenies of phage genes revealed a mosaic pattern, consistent with repeated recombination of phage genes within and across the supergroup boundary.